Influence of Estrogen on Sugar Metabolism in Totally Depancreatized Dogs

AUTHOR(S):

MIWA, TETSUO

CITATION:

MIWA, TETSUO. Influence of Estrogen on Sugar Metabolism in Totally Depancreatized Dogs. 日本外科宝函 1968, 37(1): 58-75

ISSUE DATE:

1968-01-01

URL:

http://hdl.handle.net/2433/207442

Influence of Estrogen on Sugar Metabolism in Totally Depancreatized Dogs

by

TETSUO MIWA

From the 1st Department of Surgery Kyoto University Medical School
(Director : Prof. Dr. ICHIO HONJO)

Received for Publication Oct. 27, 1967

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I. INTRODUCTION

Among various types of experimentally produced diabetes, diabetes produced by total pancreatectomy has the longest history and this measure has been the most reliable method to produce experimental diabetes. In 1889, MEHRING and MINKOWSKI[1] discovered that pancreatic diabetes mellitus develops rapidly after total pancreatectomy with such clinical findings as hyperglycemia, glycosuria, thirst and ketosis, and with this discovery they largely contributed to the understanding of diabetes mellitus in humans.

Recent advance in surgery has made it feasible to remove the entire pancreas in cases of malignant neoplasms in this organ. However, pathophysiologic alteration after total pancreatectomy is so complicated that many problems still remain to be clarified despite numerous studies have been made in this field.

In his investigation on changes of the sexual gland in totally depancreatized dogs, NISHIKAWA[2] discovered, in 1963, that general condition was maintained favorably and weight loss was also mild in parallel with increase in urinary estrogen, when G. T. H. (Gonadotropic Hormone) was administered into female dogs, as compared with animals

treated with insulin alone.

The author of the present paper studied pathophysiologic change in totally depancreatized dogs treated with estrogen from the aspect of sugar metabolism.

II. MATERIALS AND METHODS

A. Materials

As the experimental animals, adult mongrel dogs of both sexes of the same number were used. Total pancreatectomy was performed as described in the below on these animals.

B. Methods

1. Total pancreatectomy

After 12 hours' fasting state, 50 mg of Cocktelin H was intramuscularly injected and from 10 to 20 mg per kg body weight of Isozol was intravenously injected 30 minutes later. Thus, the animals were anesthetized and the abdomen was opened. Since the duodenum and the pancreas are not fixed to the posterior wall of the abdominal cavity in dogs, total pancreatectomy can be carried out relatively easily, as long as the isolation of tight fixation between the duodenal wall and the body of the pancreas be carefully performed after the ligation of the small vessels to the pancreas with care not to injure the pancreaticoduodenal artery and vein.

2. Groups of experimental animals

Totally depancreatized dogs were divided into the following four groups according to the schedule of insulin and estrogen administration.

(1) Group without administration of drug. This group was consisted of animals receiving neither insulin nor estrogen.

(2) Group of estrogen administration alone (abbreviated to estrogen group, hereafter). Animals of this group received estrogen administration alone after total pancreatectomy.

(3) Group of insulin administration (abbreviated to insulin group, hereafter). These animals were treated with insulin alone after surgery.

(4) Group of simultaneous administration of insulin and estrogen (abbreviated to insulin-estrogen group, hereafter). Animals in this group were administered simultaneously with insulin and estrogen after surgery.

Besides these four groups, normal animals were used for control studies.

At insulin administration, 1 to 2 units per kg body weight of crystalline insulin were intramuscuarly injected two times a day.

For estrogen administration, 500 to 2000 units per kg body wright of Estradiol-Benzoate were administered every day starting from 2nd postoperative day.

3. Measurement of body weight

In the fasting state in the early morning, body weight of totally depanoreatized dogs was measured.

4. Determination of blood sugar level and urinary sugar level

Twenty-four hours' total urine was collected and blood was taken in the fasting state in the early morning on the next day for the determination of blood sugar and urinary sugar levels, which was performed employing electric colorimetric quantitative determina-

tion of SOMOGYI-NELSON[3].

5. Determination of insulin sensitivity and assimilation index

In the fasting state in the early morning, 0.25 units per kg body weight of crystalline insulin were intravenously injected in the animals of each group. Blood sugar level was determined every 30 minutes after the injection for 9 hours. For the determination of assimilation index, method of NORGAARD-THAYSEN[4] was employed.

6. Determination of blood sugar level after intravenous administration of estrogen

In order to investigate whether or not estrogen has direct influence on blood sugar level, 1000 units per kg body weight of estrogen was intravenously administered and blood sugar level was determined every 1 hour.

7. Determination of glycogen content in the liver

With the care of minimizing operative aggression, the abdomen of experimental animals was opened every 1 week after surgery and small section of the liver was taken for the determination of glycogen content. The determination was performed by electric colorimetric quantitative determination of GOOD-KAMER and SOMOGYI.[5]

8. Determination of acetone body in blood and urine

In the blood taken in the fasting state in the early morning and in 24 hours' total urine, acetone body was determined. For the determination, modified method of GREENBERG and LESTER and electric colorimetric quantitative determination of KOIDE, KOOYAWA and MORITA[6] were used.

III. RESULTS

1. Survival time and rate of weight loss after total pancreatectomy

The experimental animals showed hyperglycemia and glycosuria as early as 24 hours after total pancreatectomy. If any drugs were not administered, characteristic signs of pancreatic diabetes such as thirst, polyuria and steatorrhea with gradual weight loss developed. Survival time of these animals was, as summarized in Tab. 1, approximately 2 weeks after surgery.

When estrogen alone was administered after surgery, animals could survive more than 3 weeks, as shown in Tab. 2. In animals treated with insulin alone after surgery, and if properly administered, survival time was longer than 4 weeks as shown in Tab. 3. When insulin and estrogen were simulaneously administered, the animals, as shown in Tab. 4, could survive more than 5 weeks after surgery with improvement of thirst, polyuria and steatorrhea, and the animals of this group showed the most favorable postoperative course than any other groups without signs of development of fatty liver.

Rate of weight loss, as shown in Tab. 5, 6, 7 and 8 and Fig. 1, was 30.1 per cent in the group without administration of drug, 17.0 per cent in estrogen group, 17.4 per cent in insulin group and 6.2 per cent in insulin-estrogen group, respectively 2 weeks after total pancreatectomy. Rate of weight loss was obviously the smallest in insulin-estrogen group.

2. Fluotuation in blood sugar level and urinary sugar level

Blood sugar level in the fasting state in the early morning reached its maximum level from 24 to 48 hours after total pancreatetcmy in dogs, which was followed by continuous hyperglycemia. Fluctuation in blood sugar level in the fasting state in the early morning

Table 1 Survival Time after Total Pancreatectomy in Group without Administration of Drug

Dog No.	Sex	Survival Time (day)
No. 1	♀	14
No. 2	♂	6
No. 3	♀	12
No. 4	♂	11
No. 5	♀	9
No. 6	♂	7
Mean (day)		10

Table 2 Survival Time after Total Pancreatectomy in Estrogen Group

Dog No.	Sex	Survival Time (day)	Dosis of Estrogeln (u/kg)
No. 1	♀	24	10000
No. 2	♀	6	20000
No. 3	♀	26	10000
No. 4	♂	5	20000
No. 5	♂	12	5000
No. 6	♀	21	5000
No. 7	♀	23	5000
No. 8	♂	18	4000
No. 9	♂	106	1000
No. 10	♂	30	1000
No. 11	♂	27	1000
No. 12	♂	35	1000
No. 13	♂	28	1000
No. 14	♀	29	1000
No. 15	♀	45	1000
No. 16	♀	19	1000
Mean (day)		28	

Table 3 Survival Time after Total Pancreatectomy in Insulin Group

Dog No.	Sex	Survival Time (day)	Dosis of Insulin (u./kg)
No. 2	♂	46	1.5
No. 3	♀	40	1.5
No. 5	♀	31	1.5
No. 6	♂	28	2.0
Mean (day)		36	

Table 4 Survival Time after Total Pancreatectomy in Insulin-Estrogen Group

Dog No.	Sex	Survival Time (day)	Dosis of Insulin (u./kg)	Dosis of Estrogen (u./kg)
No. 1	♂	18	1.0~2.0	2000
No. 2	♂	50	1.0~2.0	1000
No. 3	♂	131	1.0	1000
No. 4	♂	23	1.0	1000
No. 5	♂	34	1.0	2000
No. 6	♂	49	1.0	1000
No. 7	♀	63	1.0	1000
No. 8	♀	29	1.0	1000
No. 9	♂	37	2.0	1000
No. 10	♀	53	1.0	1000
No. 11	♂	98	1.0	1000
Mean (day)		53		

Table 5 Rate of Weight Loss in Group without Administration of Drug

Dog No.	Sex	Body Weight (kg)	after Surgery (week)	
			1	2
No. 1	♀	8.0	18.8 %	31.3 %
No. 2	♂	8.0	25.0	—
No. 3	♀	12.5	16.0	—
No. 4	♂	11.5	17.4	30.4
No. 5	♀	10.5	14.3	28.6
Mean (%)			18.3	30.1

Table 6 Rate of Weight Loss in Estrogen Group

Dog No.	Sex	Body Weight (kg)	after Surgery (week)			
			1	2	3	4
No. 1	♀	8.0	6.3%	12.5%	15.0	—
No. 3	♀	11.0	9.1	18.2	13.6	—
No. 6	♀	13.0	7.7	19.2	27.0	—
No. 7	♀	7.5	13.3	6.7	20.0	—
No. 9	♂	9.5	0	0	-5.3	0
No. 10	♂	12.5	8.0	12.0	16.0	—
No. 11	♂	14.5	13.8	31.0	35.7	—
No. 12	♂	14.0	7.1	14.3	21.4	36.8
No. 13	♂	10.5	4.8	9.5	14.3	—
No. 14	♀	8.0	12.5	25.0	37.5	37.5
No. 15	♀	14.0	10.7	21.4	35.7	39.3
Mean (%)			9.3	17.0	23.6	37.9

Table 7 Rate of Weight Loss in Insulin Group

Dog No.	Sex	Body Weight (kg)	after Surgery (week) 1	2	3	4
No. 2	♂	9.5	10.5%	21.1%	26.3	31.6
No. 3	♀	8.5	5.9	11.8	17.6	23.5
No. 4	♂	12.5	8.0	12.0	16.0	—
No. 5	♀	9.5	10.5	21.1	26.3	33.7
No. 6	♂	12.0	12.5	20.8	29.2	33.3
Mean (%)			9.5	17.4	23.1	25.5

Table 8 Rate of Weight Loss in Insulin-Estrogen Group

Dog No.	Sex	Body Weight (kg)	after Surgery (week) 1	2	3	4
No. 2	♂	15.0	6.7%	6.7%	13.3%	16.7%
No. 3	♂	9.0	5.6	5.6	5.6	16.7
No. 4	♂	13.0	0	7.7	11.5	—
No. 6	♂	10.0	10.0	5.0	10.0	15.0
No. 7	♀	9.0	0	5.6	11.1	11.1
No. 8	♀	10.0	5.0	5.0	25.0	25.0
No. 9	♂	11.5	4.3	7.0	8.7	8.7
No. 10	♀	12.5	4.0	8.0	16.0	24.0
No. 11	♂	12.5	4.0	5.6	8.0	8.0
Mean (%)			4.4	6.2	12.1	15.7

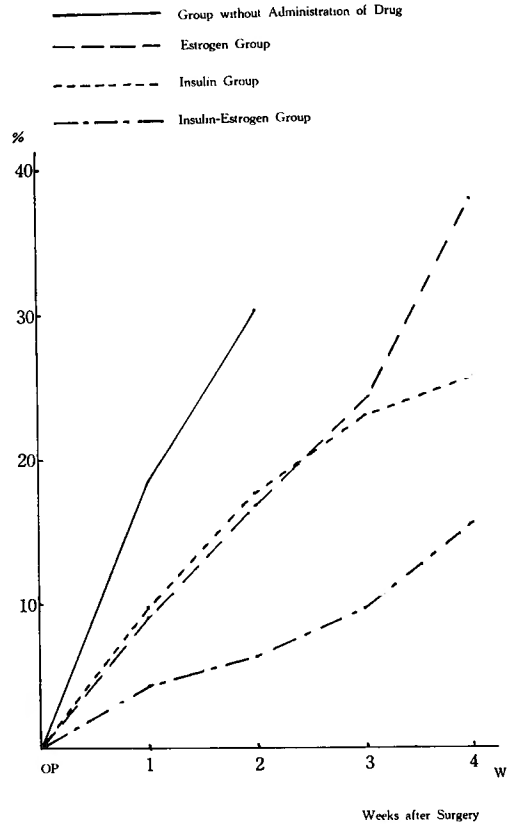

Fig. 1 Rate of Weight Loss

is summarized in Tab. 9, 10, 11 and 12 and Fig. 2. Namely, in group without administration of drug, it remained in a hyperglycemic level of 350 to 400 mg/dl, whereas in estrogen group, insulin group and insulin-estrogen group, it was invariably maintained in a level around 300 mg/dl until the end of the first postoperative week, which was followed by slight increase or decrease with less significnace.

Fluctuation in urinary sugar level reached its peak from 24 to 48 hours after surgery in general, as shown in Tab. 13, 14, 15 and 16 and Fig. 3. In group without administration of drug, it was maintained in a level of glycosuria around 7.0 g/dl corresponding to the level of blood sugar in this stage. Towards the 2nd week after surgery, urinary sugar fluctuated from 5.0 g/dl to 6.0 g/dl in insulin group, while in estrogen group and insulin-estrogen group, it ranged between 2.0 g/dl to 4.0 g/dl despite hyperglycemia in this stage.

In brief, urinary sugar level showed a tendency of decrease after total pancreatectomy

Table 9 Blood Sugar Level after Total Pancreatectomy in Group without Administration of Drug

Dog No.	Sex	before Surgery	after Surgery (day) 1	2	4	7	10	14
No. 1	♀	104	262	386	394	390	402	314
No. 3	♀	76	396	372	340	384	376	—
No. 4	♂	92	342	354	338	362	344	—
Mean (mg/dl)		91	333	370	358	379	374	314

Table 10 Blood Sugar Level after Total Pancreatectomy in Estrogen Group

Dog No.	Sex	before Surgery	after Surgery (day) 1	2	4	7	10	14	17	21	24	28
No.1	♀	76	208	246	228	302	300	342	400	408	364	—
No.3	♀	106	304	360	364	298	316	296	270	182	206	—
No.6	♀	90	282	306	360	344	322	308	212	204	—	
No.7	♀	88	286	380	312	236	120	100	124	120	—	—
No.8	♂	102	342	386	378	360	364	376	248	—	—	—
No.9	♂	90	320	344	296	300	262	246	248	268	316	370
No.10	♂	84	294	292	274	254	268	360	352	338	344	320
Mean (mg/dl)		91	291	330	316	299	279	290	265	254	308	354

Table 11 Blood Sugar Level after Total Pancreatectomy in Insulin Group

Dog No.	Sex	before Surgery	after Surgery (day) 1	2	4	7	10	14	17	21	24	28	35
No. 2	♂	88	296	422	392	344	404	394	422	354	406	420	438
No. 3	♀	72	306	342	406	294	350	418	460	388	330	322	308
No. 5	♀	92	262	288	178	252	314	292	318	326	294	344	—
Mean (mg/dl)		84	288	352	326	298	356	368	400	356	344	362	374

Table 12 Blood Sugar Level after Total Pancreatectomy in Insulin-Estrogen Group

Dog No.	Sex	before Surgery	after Surgery (day) 1	2	4	7	10	14	17	21	24	28	35
No. 2	♂	78	346	360	264	380	370	260	248	426	460	434	384
No. 3	♂	84	174	212	386	324	346	254	398	382	422	402	424
No. 4	♂	86	354	368	352	310	154	222	264	212	—	—	
No. 5	♂	88	202	234	206	198	142	140	136	168	136	118	—
No. 6	♂	70	314	380	358	330	280	196	182	208	214	176	168
No. 7	♀	74	380	356	372	340	328	366	392	422	388	312	286
Mean (mg/dl)		80	295	318	313	314	270	240	270	308	324	288	316

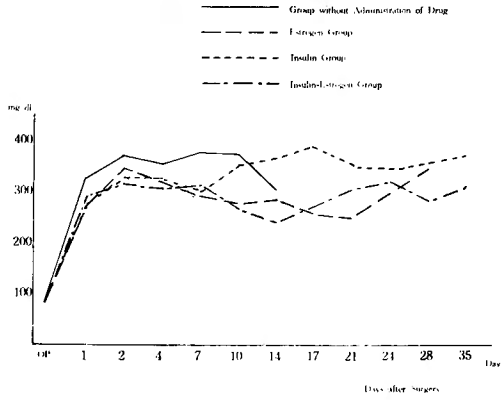

Fig. 2 Blood Sugar Level after Total Pancreatectomy

Table 13 Urinary Sugar Level after Total Pancreatectomy in Group without Administration of Drug

Dog No.	Sex	before Surgery	after Surgery (day) 1	2	4	7	10	14
No. 1	♀	0.3	7.5	8.0	7.6	6.8	7.5	6.0
No. 3	♀	0.1	6.5	6.8	6.2	7.0	5.4	4.2
No. 4	♂	0.1	7.5	6.5	5.8	6.5	7.0	—
Mean (g/dl)		0.17	7.2	7.1	6.5	6.8	6.6	5.1

Table 14 Urinary Sugar Level after Total Pancreatectomy in Estrogen Group

Dog No.	Sex	before Surgery	after Surgery (day) 1	2	4	7	10	14	17	21	24	28
No.1	우	0.1	6.2	5.8	4.6	5.5	5.0	6.2	7.5	5.4	4.2	—
No.3	우	0.4	7.3	6.2	5.8	3.0	4.2	3.6	4.4	2.4	2.8	—
No.6	우	0.5	5.8	6.4	3.2	2.8	4.2	3.3	2.5	4.1	—	—
No.7	우	0.3	5.9	6.2	2.4	1.8	0.2	0.1	0.6	0.3	—	—
No.8	숫	0.2	6.0	7.5	4.9	3.8	2.6	2.9	1.8	—	—	—
No.9	숫	0.1	2.5	3.2	2.1	4.3	0.3	0.4	0.7	1.2	1.6	1.8
No.10	숫	0.1	5.8	4.7	4.3	6.5	5.4	7.5	6.5	5.3	5.4	4.8
Mean (g/dl)		0.24	5.6	5.7	3.9	4.0	3.1	3.3	3.4	3.1	3.5	3.3

Table 15 Urinary Sugar Level after Total Pancreatectomy in Insulin Group

Dog No.	Sex	before Surgery	after Surgery (day) 1	2	4	7	10	14	17	21	24	28	35
No. 2	숫	0.3	7.5	6.5	5.6	7.5	7.5	5.2	4.8	2.6	5.8	6.2	3.6
No. 3	우	0.2	5.3	5.0	6.8	4.2	3.8	5.8	6.5	7.0	4.2	3.8	4.3
No. 5	우	0.1	5.5	4.0	3.6	5.6	7.2	4.0	6.2	5.6	3.4	4.2	—
Mean (g/dl)		0.2	6.1	5.2	5.3	5.8	6.2	5.0	5.8	5.1	4.5	4.7	4.0

Table 16 Urinary Sugar Level after Total Pancreatectomy in Insulin-Estrogen Group

Dog No.	Sex	before Surgery	after Surgery (day) 1	2	4	7	10	14	17	21	24	28	35
No. 2	숫	0.2	7.5	9.0	6.5	7.5	6.7	3.2	4.7	8.5	8.5	7.5	6.5
No. 3	숫	0.1	6.0	4.5	2.5	0.2	0.7	0.7	7.5	2.5	5.3	1.4	2.5
No. 4	숫	0.1	4.5	2.5	5.0	0.4	3.0	0.1	2.2	1.5	—	—	—
No. 5	숫	0.1	0.6	1.0	0.4	2.0	0.6	1.2	0.8	2.0	1.5	0.6	—
No. 6	숫	0.2	8.5	6.5	7.0	5.5	4.5	6.3	5.8	3.2	4.5	1.6	2.8
No. 7	우	0.3	5.4	6.3	5.0	4.0	3.6	2.2	1.4	2.4	3.2	0.8	1.2
Mean (g/dl)		0.17	5.4	5.0	4.4	3.3	3.2	2.3	3.7	3.4	4.6	2.4	3.3

by the administration of estrogen.

3. Insulin sensitivity and assimilation index

Intravenous administration of crystalline insulin in a dosis of 0.25 units per kg body weight resulted in normal dogs within 30 to 60 minutes in a marked hypoglycemia, when injected in the fasting state in the early morning, as shown in Tab. 7 and Fig. 4 and 5. The hypoglycemia recovered thereafter to the level before the injection within 2 to 3 hours. Insulin was similarly injected in the totally depancreatized animals of each group and

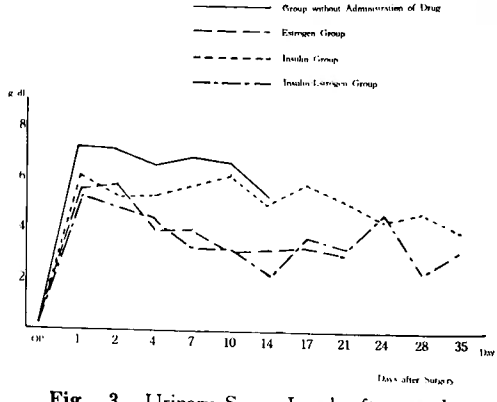

Fig. 3 Urinary Sugar Level after total Pancreatectomy

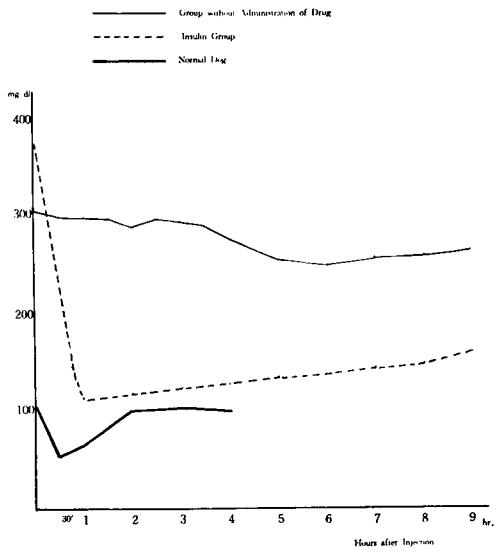

Fig. 4 Blood Sugar Level after Intravenous Injection of Insulin in Totally Depancreatized Dogs (Insulin 0. 25 u./kg)

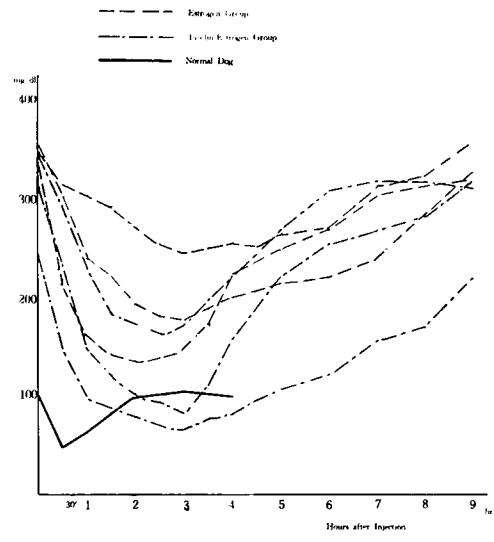

Fig. 5 Blood Sugar Level after Intravenous Injection of Insulin in Totally Depancreatized Dogs (Insulin 0. 25 u./kg)

blood sugar level was determined. As shown in Tab. 17 and Fig. 4, blood sugar level in animals without adminstration of drug falled gradually reaching the minimum value around 6 hours after intravenous injection of insulin, and then, restored gradually to the evel before the injection approximately 24 hours after the injection. In insulin group, as shown in Tab. 17 and Fig. 5, blood sugar level markedly falled from 1 to 2 hours after intravenous injection of insulin, which was followed by gradual increase to return to the level before the injection from 12 to 24 hours later. In contrast to these findings, in estrogen group and insulin-estrogen group, blood sugar level reached its minimum from 2 to 4 hours after intravenous injection of insulin, which showed rapid recovery thereafter,

Table 17 Blood Sugar Level after Intravenous Injection of Insulin in Totally Depancreatized Dogs (Insulin 0. 25 u./kg)

	before	after Surgery (hour)												
	Surgery	3. 0mm	1	1. 5	2	2. 5	3	3. 5	4	5	6	7	8	9
Normal Dog	104	52	66	86	100	102	106	104	102					
Without Admin -istration of Drugs	306	302	300	296	290	298	294	288	272	256	250	258	260	266
Insulin Administration	376	242	114	116	120	120	124	128	128	134	138	146	150	162
Estrogen Group	352	320	312	298	274	258	250	254	260	268	276	320	332	364
	342	226	164	146	138	142	150	176	228	254	274	312	322	330
	358	304	242	224	196	186	180	192	204	220	224	246	292	334
Insulin-Estrogen Group	252	152	98	88	82	74	68	78	84	110	128	160	174	224
	318	240	148	126	104	96	84	112	160	226	260	274	288	324
	352	306	238	188	142	164	176	202	224	272	316	328	324	316

returning to the pre-injection level 6 to 9 hours later.

To summarize these findings, although interval of time between insulin injection and manifestation of hypoglycemia was slightly prolonged compared with normal animals, interval of time for restoration of the level of blood sugar to that before the insulin injection was shortened in estrogen group and insulin-estrogen group compared with that in group without administration of drug and in insulin group, and particularly the curve of blood sugar level in insulin-estrogen group showed the likewise pattern with that in normal animals.

From these curves, assimilation index was calculated following the method of Norgaad-Thaysen as summarized in Tab. 18 and Fig. 6. It was assumed that assimilation index was smaller in totally depancreatised animals than in normal ones in the turn of insulin-estrogen group, insulin group, estrogen group and group without administration of drug, showing the smallest value in the last group.

4. Fluctuation in blood sugar level after intravenous administration of estrogen

In order to investigate whether estrogen has the effect of reducing the blood sugar content or not, 1000 units per kg body weight of estrogen was intravenously administered in group without administration of drug and estrogen group 1 to 2 weeks after total pancratectomy, and behavior of blood sugar level was pursued for 5 hours. As shown in Tab. 19 and Fig. 7, decrease in blood sugar level could not be observed by estrogen administration.

5. Rate of decrease in hepatic glycogen content

To investigate the fluctuation in hepatic glycogen content in totally depancreatized dogs, the abdomen was opened in the fasting state in the early morning and a small section of the liver was cut off for the determination of hepatic glycogen content. Decrease in hepatic glycogen content was marked in group without administration of drug, and estrogen group 1 week after total pancreatec-

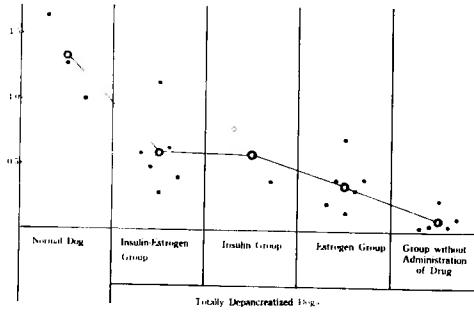

Fig. 6 Assimilation Index after Total Pancreatectomy

Table 18 Assimilation Index after Total Pancreatectomy

Normal Dog	Totally Depancreatized Dogs			
	Insulin-Estrogen Group	Insulin Group	Estrogen Group	Group without Administration of Drug
1.667	0.569	0.794	0.230	0.039
1.328	1.155	0.526	0.401	0.048
1.012	0.306	0.398	0.314	0.050
	0.493		0.417	0.232
	0.647		0.169	0.113
	0.416		0.714	
1.335	0.598	0.573	0.374	0.096

tomy, the rate of decrease being 94.7 per cent in the former and 88.1 per cent in the latter, as shown in Tab. 20 and 21 and Fig. 8, while the rate of decrease was as slight as 24.2 per cent in insulin group and 27.5 per cent in insulin-estrogen group. Thus, the decrease in hepatic glycogen content was found to be far smaller in the cases of insulin administration. Rate of decrease in hepatic glycogen content was 94.9 per cent in group without administration of drug 2 weeks after surgery and it was presumed that there was no storage of glycogen within the liver. Three

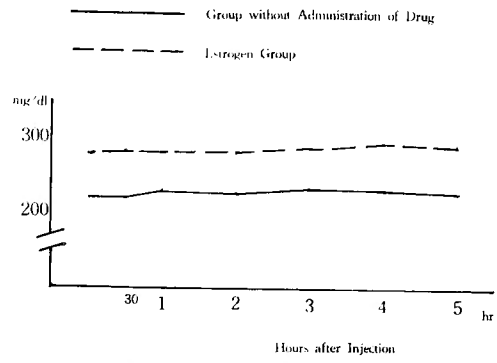

Fig. 7 Blood Sugar Level after Intravenous Injection of Estrogen in Totally Depancreatized Dogs

Table 19 Blood Sugar Level after Intravenous Injection of Estrogen in Totally Depancreatized Dogs (1 to 2 weeks after Total Pancreatectomy, Estrogen 1,000 u./kg)

	Sex	Body Weight (kg)	before Injection	after Injection (hour) 30min.	1	2	3	4	5
Group without Administration of Drug	♀	10.5	224	220	232	228	236	234	226
Estrogen Group	♂	10.5	280	284	282	280	286	296	292

Table 20 Fluctuation of Hepatic Glycogen Content after Total Pancreatectomy

	Dog No.	Sex	Body Weight (kg)	before Surgery	after Surgery (week) 1	2	3
Group without Administration of Drugs	No. 3	♀	12.5	3910	186	378	—
	No. 4	♂	11.5	6812	124	210	—
	No. 5	♀	10.5	5832	280	182	—
	Mean (mg/dl)			5518	297	257	—
Insulin Group	No. 4	♂	12.5	6380	7216	—	3116
	No. 5	♀	9.5	3932	2106	—	1618
	No. 6	♂	12.0	5010	3120	—	2208
	Mean (mg/dl)			5107	4147	—	2314
Estrogen Group	No. 10	♂	12.5	2102	385	—	590
	No. 12	♂	14.0	3460	286	—	627
	No. 15	♀	14.0	2596	236	—	812
	Mean (mg/dl)			2753	303	—	676
Insulin-Estrogen Group	No. 7	♀	9.0	4287	4480	—	1972
	No. 9	♂	11.5	4168	2630	—	2261
	No. 11	♂	12.5	4341	2175	—	4608
	Mean (mg/dl)			4265	3095	—	2947

Table 21 Rate of Decrease in Hepatic Glycogen Content after Total Pancreatectomy

	Dog No.	Sex	Body Weight (kg)	before Surgery	after Surgery (week) 1	2	3
Group without Administration of Drugs	No. 3	♀	12.5	3910	95.2	90.3	—
	No. 4	♂	11.5	6812	93.8	97.0	—
	No. 5	♀	10.5	5832	95.2	96.9	—
	Mean (%)			5518	94.7	94.9	—
Insulin Group	No. 4	♂	12.5	6380	−11.6	—	51.2
	No. 5	♀	9.5	3932	46.4	—	58.9
	No. 6	♂	12.0	5010	37.7	—	55.9
	Mean (%)			5107	24.2	—	55.3
Estrogen Group	No. 10	♂	12.5	2102	81.6	—	71.9
	No. 12	♂	14.0	3460	91.7	—	81.9
	No. 15	♀	14.0	2596	90.9	—	68.7
	Mean (%)			2753	88.1	—	74.2
Insulin-Estrogen Group	No. 7	♀	9.0	4287	4.5	—	54.0
	No. 9	♂	11.5	4168	37.0	—	45.8
	No. 11	♂	12.5	4341	49.9	—	−5.8
	Mean (%)			4265	27.5	—	31.3

weeks after surgery, the rate of decrease was 74.2 per cent in estrogen group being smaller than the value in this group 1 week after surgery while in insulin group, the rate decreased on to be 55.3 per cent 3 weeks after surgery. The rate of decrease was considerably smaller in insulin-estrogen group than in any other groups being 31.3 per cent 3 weeks after surgery.

6. Fluctuation in acetone body in blood and urine

Acetone body in blood and urine was determined simultaneously with the determination of blood and urinary sugar levels in each group after total pancreatectomy. As shown in Tab. 22 and Fig. 9, acetone body in blood rapidly increased after surgery in every group, reaching the maximum towards the end of 1st postperative week, and decreased gradually thereafter. Acetone body content was 0.9 mg/dl 4 days after surgery and reached the maximum of 1.1 mg/dl 1 week

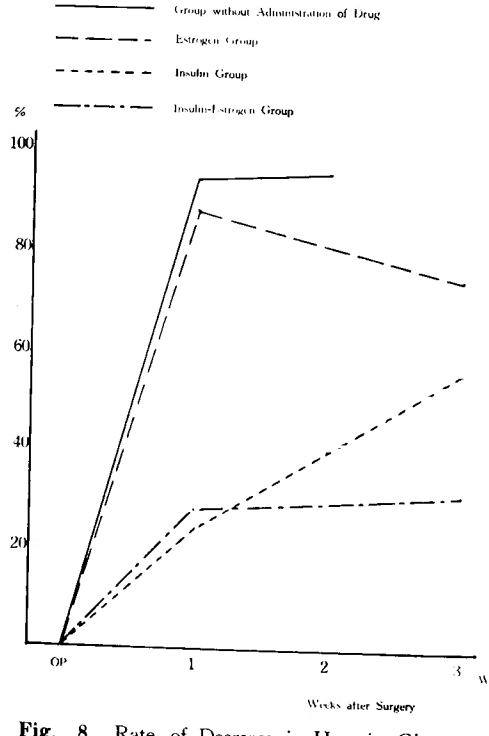

Fig. 8 Rate of Decrease in Hepatic Glycogen Content after Total Pancreatectomy

Table 22 Fluctuation of Blood Acetone Body after Total Pancreatectomy

	Dog No.	Sex	Body Weight (kg)	after Surgery (week) 4 day	1	2	3	4
Group without Administration of Drug	No. 1	♀	8.0	1.0	1.2	0.8	—	—
	No. 4	♂	11.5	0.8	1.5	0.8	—	—
	No. 5	♀	10.5	1.0	0.7	0.6	—	—
	Mean (mg/dl)			0.9	1.1	0.7	—	—
Insulin Group	No. 3	♀	8.5	0.5	0.2	0.3	0.1	0.1
	No. 5	♀	9.5	0.6	1.2	0.4	0.3	0.1
	No. 6	♂	12.0	1.2	1.4	0.3	0.3	0.2
	Mean (mg/dl)			0.8	0.9	0.3	0.2	0.1
Estrogen Group	No. 13	♂	10.5	1.8	1.2	1.8	0.8	—
	No. 14	♀	8.0	1.2	1.2	0.8	0.6	—
	No. 15	♀	14.0	1.5	4.0	1.9	0.6	—
	Mean (mg/dl)			1.5	2.1	1.5	0.7	—
Insulin-Estrogen Group	No. 8	♀	10.0	1.4	3.0	1.7	0.3	0.3
	No. 9	♂	11.5	0.7	0.5	0.8	0.4	0.4
	No. 11	♂	12.5	1.3	1.9	1.6	0.9	1.2
	Mean (mg/dl)			1.1	1.8	1.4	0.5	0.6

after surgery in group without administration of drug which was followed by gradual decrease. However, the content remained in a higher level until terminal stage, ranging around 0.7 mg/dl 2 weeks after surgery. In insulin group, the content ranged a little lower than in group without administration of drug, showing a rapid decrease to be 0.3 mg/dl 2 weeks after surgery, followed by gradual decrease there after. In estrogen group, the content increased instantaneously, showing 1.5 mg/dl on the 4th day after surgery and reaching the highest value of 2.1 mg/dl 1 week after surgery. It showed about two times as high a value as that in group without administration of drug to be 1.5 mg/dl 2 weeks after surgery, and continued to maintain its relatively high value of 0.7 mg/dl 3 weeks after surgery. In insuin-estrogen group, the content ranged slightly lower than in estrogen group until 3 weeks after surgery, and it slightly increased to be 0.6 mg/dl towards the 4th week after surgery. It is assumed that

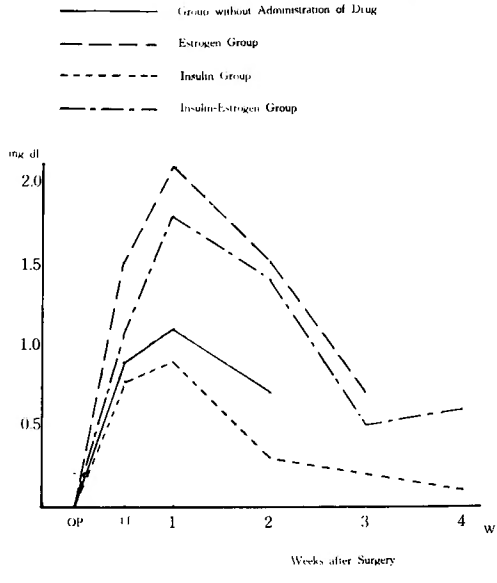

Fig. 9 Fluctuation of Blood Acetone Body after Total Pancreatectomy

there was an obvious tendency of increase in acetone body in blood by the administration of estrogen after total pancreatectomy.

Urinary acetone body increased in parallel with the increase in blood acetone body, reaching its maximum level 7 days after surgery in all groups and followed by gradual decrease thereafter, as shown in Tab. 23 and Fig. 10. In group without administration of drug, the content was 43 mg/day 4 days after surgery, and it reached the maximum level of 55 mg/day 7 days after surgery. Although it decreased gradually thereafter, the content still remained to be 46 mg/day even 2 weeks after surgery. In insulin group, the content ranged slightly lower than in group without administration of drug, showing rapid decrease to be 21 mg/day 2 weeks after surgery. However, it still remained to be 14 mg/day even 4 weeks after surgery. In contrast to these findings, urinary acetone body content increased rapidly after surgery in estrogen group, ranging to be 64 mg/day 4 days after surgery and it reached its maximum value of

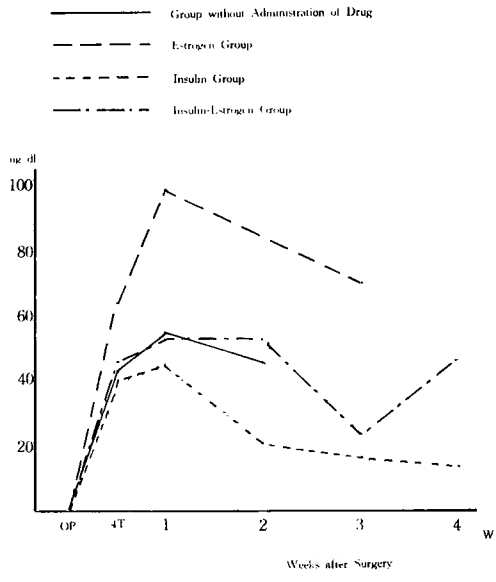

Fig. 10 Fluctuation of Urinary Acetone Body after Total Pancreatectomy

Table 23 Fluctuation of Urinary Acetone Body after Total Pancreatectomy

	Dog No.	Sex	Body Weight (kg)	after Surgery (week) 4 day	1	2	3	4
Group without Administration of Drug	No. 1	♀	8.0	48	54	42	—	—
	No. 4	♂	11.5	22	34	24	—	—
	No. 5	♀	10.5	60	76	72	—	—
	Mean (mg/day)			43	55	46	—	—
Insulin Group	No. 3	♀	8.5	38	26	14	16	18
	No. 5	♀	9.5	22	50	18	16	10
	No. 6	♂	12.0	64	56	32	20	14
	Mean (mg/day)			41	45	21	17	14
Estrogen Group	No. 13	♂	10.5	64	76	118	112	—
	No. 14	♀	8.0	46	62	42	34	—
	No. 15	♀	14.0	82	158	100	64	—
	Mean (mg/day)			64	99	87	70	—
Insulin-Estrogen Group	No. 8	♀	10.0	36	50	34	28	26
	No. 9	♂	11.5	26	12	16	10	24
	No. 11	♂	12.5	74	100	110	106	90
	Mean (mg/day)			45	54	53	23	47

99 mg/day 7 days after surgery. It decreased gradually thereafter, but it remained in a higher level of 87 mg/day even 3 weeks after surgery, corresponding 2 to 3 times higher than in any other groups.

In insulin-estrogen group, the content was slightly higher than in group without administration of drug until 2 weeks after surgery, which decreased rapidly towards the 3rd week postoperatively, and it increased again slightly in the 4th postoperative week to be 47 mg/day.

Thus, urinary acetone body obviously increased by the administration of estrogen after total pancreatectomy, as acetone body in blood did.

IV. DISCUSSION

Since the disocovery of insulin by BANTING[7] in 1922, effect of insulin on totally depancreatized animals has been investigated, and it was clarified that simple administration of insulin cannot keep totally depancreatized animals alive, owing to the development of fatty liver, and faty liver can be prevented by feeding the animals with diet containing raw pancreas. HERSHY[8] reported, in 1930 and 1931, that yolk lecithin also had similar effect, and BEST[9] and others also asserted in 1932 and 1935, the similar efficiency of betaine and choline. In 1936, however, DRAGSTEDT[10] maintained that the effectiveness of raw pancreas in preventing the development of fatty liver is not due to choline or pancreatic enzyme but due to a new pancreatic hormone "lipocaic". On the other hand, RALLI[11], in 1938, and CHAIKOFF,[12] in 1939, demonstrated the development of fatty liver by ligation of the pancreatic duct, and emphasized an imporftant role of pancreatic juice itself in preventing the development of fatty liver. In our country, HONJO[13][14] pointed out in 1954 an important fact, from his observation in which fatty liver could not be demonstrated in totally depancreatized dogs fed by boiled rice and wheat mixed with dried fish that fatty liver, which develops frequently after total pancreatectomy, is largely influenced by diet. However, postoperative steatorrhea and progressive weight loss cannot be inhibited, even if development of fatty liver can be prevented after total pancreatectomy by the administration of raw pancreas and/or methionine. This might partly be due to loss of pancreatic excretion in respect of digestion and absorption, and more largely be due to lack of insulin caused by total removal of the pancreas which leads to a decrease in sugar utilization with necessarily resulting alteration in metabolic pattern in which protein and fat must be utilized energy source within organisms.

In the present experiment, weight loss could be prevented in the animals of both sexes by the administration of estrogen after total pancreatertomy, and development of fatty liver could hardly be observed. Here arises a question how estrogen could prevent weight loss after total pancreatectomy. When estrogen is admistered in totally depancreatized animals, postoperative steatorrhea can be remarkably improved. HUKAYA[15] had interest in this point in 1966, and made an investigation on digestion and absorption in totally depancreatized dogs treated with estrogen. In his experiment, he clarified that digestion of fat was markedly improved compared with totally depancreatized dogs treated with insulin alone, as had been the case. On the other hand, in the author's experiment, any significant changes in blood sugar level could not be observed even through estrogen was directly injected intravenously in totally depancreatized dogs. Hence, it is assumed

that estrogen is not possessed of an effect of decreasing the level of blood sugar like insulin, but the effect of estrogen to inhibt weight loss after total pancreatectomy is presiumably nothing but a result of improvement in fat metabolism.

It has been pointed out that animals become more sensitive to insulin after total pancreatectomy and one is forced to reduce the insulin dosage[16]. KAWAMURA[17] postulated that the necessity of reduction of insulin dosage is not due to increased sensitivity to insulin, but due to general consumption caused by steatorrhea, and that there is no need to reduce insulin dosage as far as appetite of the animals is maintained well.

According to the test of insulin sensitivity in the present experiment, blood sugar reached ones its minimum level after intravenous injection of insulin and it was ascertained that subsequent restoration of blood sugar level in group without administration of drug and insulin group was obviously delayed compared with that in estrogen group, and in the former groups, experimental animals frequently fell into shock after prolonged hypoglycemia. On the contrary, restoration from hypoglycemia was far shortly attained in estrogen group compared with any other groups, and hypoglycemic shock could be scarecely observed. This finding was interpreted to sustain Kawamura's presumption together with the fact, as observed by Fukaya, that digestion and absorption of fat is markedly improved after total pancreatectomy by the administration of estrogen compared with simple administration of insulin. On the other hand, there exists intimate correlation between insulin sinsitivity and hypophyseal function as maintained by BODE[18] in 1950. He observed that insulin dosage had to be gradually decreased in the experiment of insulin sensitivity in hypophysectomized dogs, and he clarified that insulin sensitivity could be maintained to be normal, if GH (Growth Hormone) had been administered in experimental dogs. HATTA [19] reported in 1965 that anterior pituitary cells were favorably preserved by administration of estrogen in totally depancreatized dogs, as compared with those without administration of drugs and those treated with insulin, suggesting favorable influence of estrogen on these cells. Hence it is assumed that prompt restoration of blood sugar level in estrogen group at insulin sensitivity test is presumably due to the fact that hypophyseal function is favorably preserved by administration of estrogen.

YAO[20], in 1959, could not find significant difference between assimilation index and hepatic glycogen content. In the present experiment, however, among experimental groups the order of decrease in assimilation index corresponded well with that of rate of decrease in hepatic glycogen contents. The rate of decrease in hepatic glycogen was smaller in insulin-estrogeen group than in insulin group. Effect of estrogen is recognized here also. It is interpreted that estogen does not act directly to the storage of hepatic glycogen, but it presumably acts via hypophyseal function and lipid metabolism in the periphery[21][22][24].

Relationship between diabetes and acetone body has been well known[24]. In the present experiment also, acetone body invariably increased in all animals after total pancreatectomy, but in insulin group, acetone body drew near to normal level with the postoperative course. On the contrary, acetone body increased far remarkably in estrogen group compared with group without administration of drug, and it was maintained in an increased level despite simultaneous administration of insulin.

Increase in acetone body in diabetes has been explained by prosperous decomposition of fat in organisms with resulting increase in acetone body production from acetyl-Co A

and disturbance of its utilization. TANIGUCHI[25] reported in 1966 that both blood lipids and non-esterified fatty acid increased on in totally depancreatized animals without administration of drug compared with other groups, and the increase was inhibited to the moderate degree by administration of estrogen, making little difference from group with insulin administration. From this finding, it is assumed that in animals without administration of drug after total pancreatectomy, there might presumably exist increase in lipid decomposition and disturbance of acetone body production. On the other hand, STADIE[26] reported in 1941 that production of ketone could not be observed in hypophysectomized animals and the hypophysis is necessary for ketone production. According to the observation of HATTA[19], histological findings of the anterior pituitary showed one-way process of devastation in totally depancreatized animals without administration of drug, and it was found to be kept in a favorable state by estrogen administration. Disturbance of acetone body production in animals without administration of drug after total pancreatectomy can be attributed to depression of hypophyseal function. Thus, it is assumed that increase in acetone body, observed by estrogen administration, might not be a result of disturbance of acetone body utilization, but a result of its excessive production, as pointed out by BENETT[27] in 1948, together with improvement of hypophyseal function.

To summarize all these findings in the present experiment, postoperative survival time could be prolonged and weight loss was inhibited also, even by a treatment with estrogen alone after total pancreatectomy. It was ascertained that pathophysiologic condition can be markedly improved after total pancreatectomy by simultaneous administration of estrogen in addition to traditional treatment with insulin alone.

Such an effect of estrogen administration was interpreted that estrogen does not act directly ageinst the disturbance of sugar metabolism after total pancreatectomy, but it influences on hypophyseal function and improves lipid metabolism after total pancreatectomy.

V. SUMMARY

Insulin and estrogen were administered in totally depancreatized dogs in various doses, and the postoperative pathophysiology was studied from the aspect or sugar metabolism, the animals being divided into insulin group, estrogen group, insulin-estrogen group and group without administration of drug. The obtained results are summarized as follows:

1. Survival time after total pancreatectomy can be prolonged by the administration of estrogen.

2. Weight loss was more slight in estrogen group as compared with group without administration of drug and the result was more favorable in the group of simultaneous administration of insulin and estrogen.

3. Administration of estrogen showed no particular influence on blood sugar level.

4. As investigated from fluctuation in blood sugar level in insulin sensitivity test, restoration of blood sugar level was more group with the administration of insulin alone.

5. Decrease in hepatic glycogen content was considerably inhibited by the administration of insulin. However the decrease was even more inhibited by the simultaneous administration of insulin and estrogen.

6. Acentone body in blood and urine increased by the administration of estrogen.

As has been described, the administration of estrogen after total pancreatectomy does

not influence directly on sugar metabolism, but it acts favorably on sugar metabolism through inprovement of lipid metabolism and hypophyseal function.

The gist of the present paper was reported at 8th Annual Meeting of Japanese Society of Diabetes.

Accomplishing the present paper, the author is deeply indebted to Pof. Dr. ICHIO HONJO for his valuable advices and kind supervision.

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和文抄録

膵全剔後の糖代謝に及ぼす Estrogen の影響

京都大学医学部外科学教室第1講座（指導．本庄一夫教授）

三　輪　哲　郎

Merig u. Minkowski (1889) が，膵全剔により膵性糖尿病が急速に発現する事を発見して以来，多くの研究者によつて糖尿病の病態生理が解明されてきた．

最近西川 (1963) が，膵全剔犬における性腺の変化を追求している際，雌犬に Gonadotropic Hormone を投与したところ，尿中 Estrogen の増量に平行して，従来の Insulin 投与のみの管理と比較して，術後一般状態が良好に経過し，体重の減少も少ない事実を発見した。著者は膵全剔犬に，各種単位の Insulin 及び Estrogen を投与し，夫々 Insulin 単独投与群，Estrogen 単独投与群，Insulin・Estrogen 併用投与群及び薬剤無投与群に分類し，その病態生理を糖代謝の面から比較検討し，次の結果を得た．

1. 膵全剔後の生存日数は，Estrogen 投与により延長させ得る．

2. 体重の減少は，薬剤無投与群に比し，Estrogen 単独投与群では軽度で，Insulin 及び Estrogen 併用投与は更に良好な成績を示した．

3. Estrogen 投与により，血糖値に特別の影響は認められなかつた．

4. Insulin感性 Test による血糖値の変動は，Insulin 単独投与群に比し，Estrogen単独投与群及び Estrogen・Insulin 併用投与群では血糖値の 復帰が良好である．

5. 肝 Glycogen 量の減少は，Insulin 投与によりかなり抑制されるが，Insulin 及び Estrogen の併用によりその減少は更に軽度となる．

6. 血液及び尿中 Aceton 体は，Estrogen 投与により増量する。

以上，膵全剔後の Estrogen 投与は，糖代謝に直接的な影響を与えず，脂質代謝ならびに下垂体機能を介して，糖代謝に好影響を及ぼしているものと解される．